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Mats Ekberg

ON COAGULATION AND FIBRINOLYSIS IN GLOMERULAR DISORDERS AND RENAL TRANSPLANTS

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This survey is based on the following papers:

I. M.Ekberg, I.M. Nilsson and Folke Linell. The significance of increased factor VIII in glomerulonephritis. Accepted for publication in Annals of Internal Medicine 8, 1975.

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VII. M.Ekberg, I.M.Nilsson, U.Hedner and B.Nosslin. Determination of urinary fibrin/fibrinogen degradation products by radioimmunoassay. Submitted.

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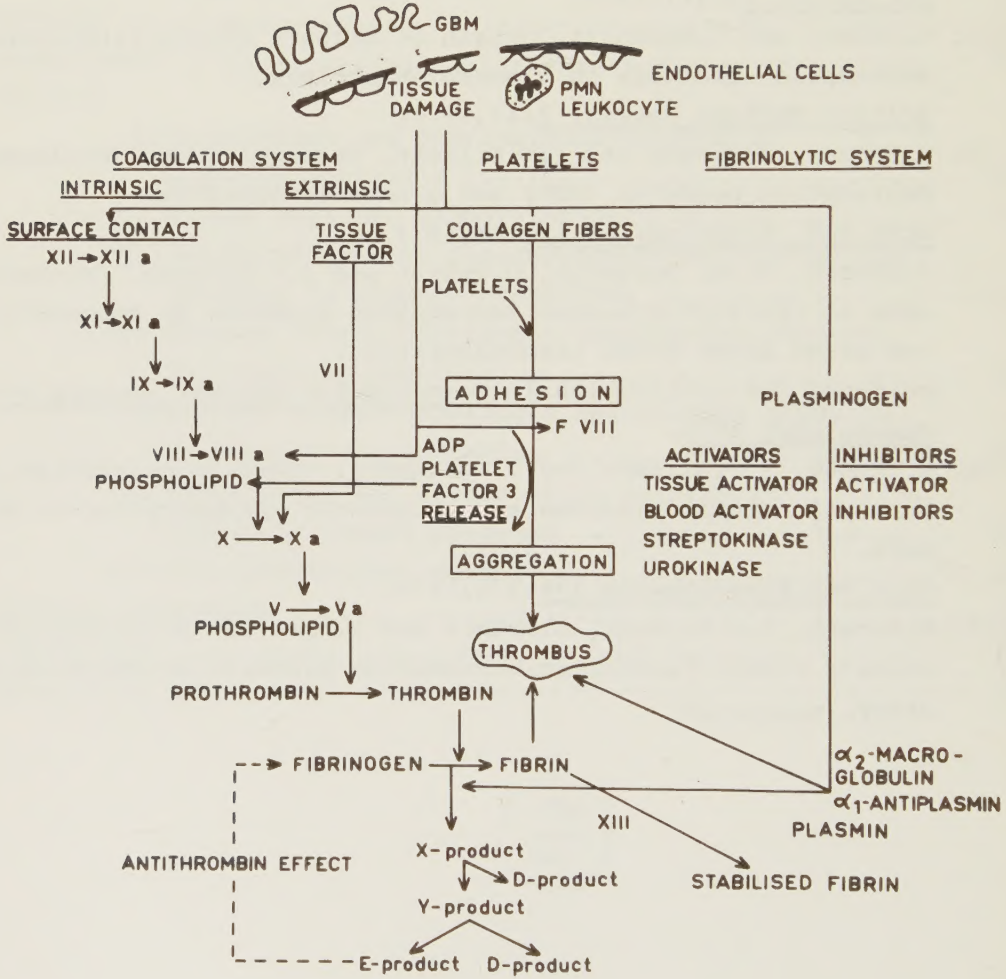


Fig. 1 Coagulation- and fibrinolytic systems

Hemostatic mechanism

Primary hemostasis following injury to vessel endothelium is initiated by adhesion of platelets to the denuded collagen fibers of the basement membrane (Hughes 1960,Jamieson et al.1971, Puett et al.1973,Jaffe et al.1974). This adhesion results in the release of various platelet factors, such as adenosin diphosphate (ADP), adrenalin, serotonin, platelet factors 3 and 4 (Hovig 1963). The platelets attached to the collagen are then covered by other platelets resulting in large aggregates, forming a loose plug. Thrombin forms with precipitation of fibrin as a result, which makes the loose platelet plug firm and impermeable. Human factor VIII can be modified in such a way as to be capable of aggregating human platelets, which indicates that factor VIII may interact in the primary hemostasis (Howard and Firkin 1971,Vermylen et al. 1973,Weiss 1975). Nachman and Jaffe (1975) have demonstrated factor VIII synthesis and secretion from cultured human endothelial cells.

The mechanism of coagulation consists of a series of enzymatic reactions, in which one coagulation factor successively activates the next (MacFarlane 1964,Davie and Ratnoff 1964,Blombäck 1966). In the intrinsic system damage to the vessel intima initiates contact-activation of factor XII, which in turn activates factor XI (Movat et al.1974). This results in the formation of a contact-activation product that activates factor IX. Activated factor IX in turn activates factor VIII, which activates factor X. In cooperation with factor V and phospholipids, factor X finally converts prothrombin to thrombin which converts fibrinogen to fibrin (Fig. 1). Movat et al.(1974) and Colman (1974) proposed that when factor XII becomes fragmented during its activation, it also gradually aquires prekallikrein activating activity. Activated factor XII has also been shown to activate the complement system (Donaldson 1968).
But prothrombin can be activated also in an extrinsic system, in which activated factor VIII is replaced by tissue factor released by damaged tissue, and a plasma factor,viz factor VII. An active product, extrinsic thromboplastin, is formed, which activates factor X (Fig. 1).

Evidence has been produced that several reactions in coagulation are mediated by adsorption of coagulation factors to phospholipids (Hemker et al.1970). There is much evidence that phospholipid adsorb activated factor X and factor V to their surface and that this complex together with Ca^{2+} is the thrombin-activating enzyme (Deggeler and Vreeken 1969). Hemker et al.(1970) demonstrated that also factor VIII and factor IX are adsorbed to a phospholipid micelle and that this complex is the active enzyme that activates factor X. Stenflo (1974) recently suggested that vitamin K is essential for the Ca^{2+} binding and thereby for the adsorption of factor IX and X to the surface of phospholipid structure like cell membrane. Prothrombin activation was also found to be accelerated by binding to phospholipid surfaces via the fragment 1 region of the prothrombin molecule (Jackson et al.1975)

Factor VIII is defined as the factor missing in classical hemophilia A. Factor VIII activity in plasma normally resides in a large macromolecule with a mol.wt. of about 2 mill (Ratnoff et al. 1969). This molecule has several well-defined properties:1)it acts in intrinsic plasma coagulation-factor VIII activity, 2) it is precipitated by specific rabbit antiserum-factor VIII antigen, 3) it probably interacts with platelets in primary hemostasis (Howard and Firkin 1971,Prentice et al.1972,Vermylen et al.1973) and is necessary to keep the bleeding time and the in vitro platelet adhesiveness normal (Bouma et al.1972)-von Willebrand factor activity and 4) it aggregates normal and von Willebrand platelets in the presence of Ristocetin (Howard and Firkin 1971,Weiss et al.1973,Holmberg et al.1974)-Ristocetin co-factor activity.
Factor VIII activity is associated with a high molecular weight protein or protein complex (Ratnoff et al.1969), which has been isolated from plasma (van Mourik et al.1970,Legaz et al.1973). No unanimity has,however, been achieved concerning the subunit structure of factor VIII. The dissociation of factor VIII from factor VIII antigen and Ristocetin co-factor activity suggests that plasma factor VIII consists of components forming a complex with all the activities. Yet SDS-gel electrophoresis after reduction of disulphide bonds shows only one band (Bennet et al.1973,

Legaz et al.1973,Holmberg et al.1974) with an estimated mol.wt. of 180-240,000, which has been taken as a sign that the protein is composed of identical subunits. The site of production of factor VIII is still debatable. Various possibilities have been suggested, such as the liver (Weaver et al.1964,Norman et al.1968, Dodds et al.1972), the RES or the blood-forming cells (Nilêhn 1962, Ponn et al.1971) and the intima of the blood vessels. Bloom et al. (1973), Hoyer et al.(1973,-74) and Holmberg et al.(1974) found a specific fluorescense in the intimal layer of the vessels in healthy persons. Jaffe et al.(1973) demonstrated synthesis of factor VIII protein by foetal endothelial cells. They recently also described factor VIII antigen synthesis and secretion from cultured human endothelial cells (Nachman and Jaffe 1975). That the presence of the antigenic protein in the intima is not due to adhesion of factor VIII from blood to the endothelial surface was also shown by Holmberg et al.(1975), who gave large transfusions of factor VIII concentrate to patients with von Willebrands disease, in which the intimal antigen is totally lacking. The factor VIII activity rose to 250% in plasma but the intima showed no specific fluorescence.

Fibrinolysis

Fibrinolysis is an enzymatic process by which fibrin/fibrinogen is broken down into smaller pieces, which are no longer clottable. Plasminogen, the inactive enzyme precursor, is converted into the proteolytic active enzyme plasmin by various activators, such as streptokinase, urokinase, tissue activators, labile blood activators and trypsin. Plasmin has a special affinity for fibrin/fibrinogen. The activation of plasminogen can be inhibited by activator inhibitors in blood or synthetic substances, such as E-aminocaproic acid and its homologues. The most important plasmin inhibitor is α_2-macroglobulin. Plasmin degrades fibrin/fibrinogen with the production of X and Y fragments, called high molecular weight degradation products (HMWDP). Further degradation yields the end products, called D and E, which are no longer coagulable, but have antigenic determinants in common with fibrin/fibrinogen. The D product inhibits fibrin polymerisation, while the E product has anti-thrombin activity.

Plasminogen is a glycoprotein normally occurring in blood plasma, other body fluids and tissues (Sherry 1968). It migrates electrophoretically with the β_2-globulin fraction of plasma (Ganrot and Nilehn 1968). The molecule is a single polypeptide chain. Its activation is a two-step sequential process. The first step involves a change of a peptide bond resulting in release of an NH_2-terminal preactivation peptide, with a mol.wt. of about 8,000. The second step consists of cleavage of a single arginyl-valine bond of the intermediate plasminogen resulting in the formation of the active enzyme plasmin (Robbins et al.1967, Walther et al. 1974). There is evidence that circulating plasminogen consists of isoenzymes with different electrophoretic properties and isoelectric points (Wallén and Wiman 1972,Summaria et al.1973). It has been shown that the native plasminogen has a glutamic acid as the N-terminal amino acid (Wallén and Wiman 1972, Rickli and Otavsky 1973,Collen 1974). The plasminogen with a lysine as the N-terminal amino acid residue, described by Robbins et al.(1967), with a mol.wt. of about 80,000 most probably represents a degraded form of glu-plasminogen (Wiman and Wallén 1973, Walther et al.1974). The glu-plasminogen described by Wallén had a mol.wt. of 90,000 and was found to consist of two different forms with differences in the amino acid sequences. Further, each of these two forms was shown to consist of 6 different isoelectric forms. Variation in the sialic acid content probably plays a role in the heterogeneity (Collen 1975).

Collen (1974) prepared undegraded plasminogen with a modification of the one-step affinity chromatography procedure of Deutsch and Merz (1970). The plasma radioactivity half-life of plasminogen with NH_2-terminal glutamic acid ranged from 2.0 to 2.5 days and the mol.wt. was 90,000, while it was less than one day for plasminogen with NH_2-terminal lysine or valine with a mol.wt. of 84,000. These findings lend support to the assumption that the native molecule consists of plasminogen with NH_2-terminal glutamic acid and a mol.wt. of 90,000.

The site of production of plasminogen is still uncertain. Some authors believe it to be the eosinophils of the bone marrow (Barnhart and Riddle 1963), but a more probable site is the liver cells (Sherry 1968, Nilsson 1971).

Plasmin is formed on activation of plasminogen. As described above, this involves the cleavage of a single arginyl-valine bond in the intermediate form of plasminogen resulting in a two-chain molecule with a heavy chain (mol.wt.55,000) and a light chain (mol.wt. 26,000), held together by a single disulphide bond (Robbins et al.1967,Robbins and Summaria 1971,Wiman and Wallén 1973). The plasmin is a serineprotease and its site of active serine and histidine is located in the light chain (Summaria et al.1973,Robbins et al.1973). Plasmin has the ability to degrade various α-amino-substituted lysine-and arginine esters (Sherry et al.1966). Plasmin splits the lysyl-lysine and lysine-arginine bonds of the fibrin(ogen) molecule (Robbins and Summaria 1971) with the production of fibrin/fibrinogen degradation products. Plasmin is not only specific for fibrinogen, but also attacks other plasma proteins, such as factor V and factor VIII (Sherry 1968,Pasquini et al.1973). Colman (1974) recently proposed that plasmin is essential for activated factor XII to acquire prekallikrein activating activity. Plasmin can also activate the complement system, in part by converting C1 to C1-esterase (Ratnoff and Naff 1967), and in part by splitting C3 and C5 resulting in release of chemotactical fragments (Ward 1967). C4 can also be inactivated by plasmin.

Activators

The conversion of plasminogen to plasmin is brought about by various activators. Plasminogen activators occur naturally in many tissues (tissue activator), in the circulating blood (blood activator) and in urine (urokinase).

Tissue activator. As early as 1947 Astrup and Permin showed that the fibrinolytic activity of tissues was due to an activator of fibrinolysis. The tissue activator has been related to the microsomes of the cells and to the lysosomes. Astrup and coworkers (1957,-66) have published extensive studies on the tissue activators. The tissue activator can be extracted by potassium thiocyanate (2M KSCN). It is thermostable (70-100°C) and is acid stable, but barely soluble in physiological saline. The localisation of fibrinolytic activity in the tissues has been facilitated by Todd's histochemical method. It is known that the tissue

activator is related chiefly to structures of the endothelial cells of the capillaries. A direct correlation has been found between the fibrinolytic activity of a tissue and its content of vascularised connective tissue. Kok and Astrup (1969) isolated plasminogen activator from hog ovaries with extraction methods and gel filtration and estimated the mol.wt. to be about 60,000. They believed it to be different from urokinase, as was also found by Kucinski et al.(1968).

In some tissues Albrectsen (1958) observed two types of plasminogen activator, an acid stable one resembling that assayed with KSCN extraction, and a labile one obtained by saline extraction. He thought that the latter might be identical with the labile blood activator. Nilsson et al.(1970) felt that endothelium of small vessels contains an activator of plasminogen, which is bound to other proteins or lipids and which has different properties when bound than when free. The activator is released in a lablie form into the blood stream, where it maintains the spontaneous fibrinolytic activity of the blood. Also Kucinski et al.(1968) felt that plasma activator is derived from tissue activator, especially that found in relation to blood vessels.

The distribution of tissue plasminogen activator has been investigated with the extraction method as well as histochemically in human and in animal tissues. Most human tissues have been found to contain tissue plasminogen activator. The highest concentrations occur in the uterus, adrenals, lymph nodes, prostate, meninges and thyroid. Little or no activity has been found in the liver, and only low activity in the renal cortex. The physiological function of these activators is apparently to dissolve fibrin precipitates in the tissues and to maintain the patency of the urinary pathways and excretory ducts.

Blood activator

Blood activator is most probably derived from the vessel walls (Kucinski et al.1968,Nilsson et al.1970). As mentioned above, the activator in the endothelium of small vessels is probably bound to other proteins or lipids and released in a labile form in the blood stream (Nilsson et al.1970). Aoki and van Kaulla (1971) prepared an activator with a mol.wt. of 65,000 from post-

mortem blood. Also Auerswald et al.(1971) studied an activator prepared from postmortem blood. The activator activity was found in protein fractions with mol.wts. of 30,500, 61,000 and 120,000. Most of the activity was found in the fraction with a mol.wt. of 61,000, and it was suggested that the vascular plasminogen activator consists of polymers of a protein with a mol.wt. of 30,500. Hampton et al.(1972) have isolated a blood activator from plasma which had a mol.wt. of 15,000-20,000 and which behaved like tissue activator on chromatography on Sephadex G-100.

Circulating blood normally contains traces of this activator (Andersson et al.1962), but various stimuli, such as physical exercise, operations, ischaemia, venous occlusion and shock raises the fibrinolytic activity in the blood (Sherry et al.1959,Hilden and Lauritsen 1971,Robertsson 1971). Also various vasoactive compound such as epinephrine, vasopressin and noradrenalin have a profound influence on the fibrinolytic activity (Sherry et al. 1959,Robertsson 1971, Mannucci 1975).

Urokinase is a protein with β-globulin mobility and normally occurs in the urine. The molecule is built up of a single polypeptide chain. Its molecular weight has been given as 53,000 (Lesuk et al.1965) and White et al.(1966) described 2 main components in the urine, one with a mol.wt. of 32,500, the other with a mol.wt. of 54,700. Urine has a high fibrinolytic activity owing to its content of urokinase. Urokinase is not identical with the blood activator (Nilsson et al.1970,Kucinski et al.1968, Hampton et al.1972) or with the tissue activator (Thorsen and Astrup 1969, Aoki and van Kaulla 1971,Astrup and Thorsen 1972). Recently, however, Bernik et al.(1974) found immunological identity between urokinase and plasminogen activator of heart, peripheral vein and kidney cultures. Much suggests that urokinase is synthetised in the kidney and possibly in the epithelium of the urinary tract (Bernik and Kwaan 1967,Åstedt and Pandolfi 1971, Åstedt 1975,Andrassay et al.1975).

Proteases of leukocytes

Human granulocytes contain large amounts of proteases with neutral pH-optimum, which have been isolated and characterised by

Ohlsson and Olsson (1973,-74). The collagenases, which are Ca^{2+}-dependent, split the collagen-molecule into two fragments, but they have also been found to have fibrinolytic activity. The elastases, giving three electrophoretic fractions with a mol.wt. of 30,000,respectively, hydrolyse not only elastin but also fibrin, kasein and low-molecular alanine esters. Odeberg et al.(1975) recently described also chymotrypsin-like enzymes in human granulocytes. Liberation of these neutral proteases can cause tissue damage, participate in the formation of chemotactical fragments through cleavage of complement factors and form kinins from kininogen.

Inhibitors of fibrinolysis

The fibrinolytic enzyme system can be inhibited at two levels, by inhibiting the effects of plasmin on fibrin or by inhibiting the plasminogen activation. Hedner (1973) purified an inhibitor of the plasminogen activation with α_2-globulin mobility and prepared a rabbit antiserum against this inhibitor fraction. After gel filtration of human serum, Aoki and van Kaulla (1971) found one fraction that inhibited plasminogen activation by blood activator. Activator inhibitors with mol.wts. of 43,000 (Kawano et al.1970) and 100,000 (Uszynski and Abildgaard 1971) have been prepared from placenta. Both inhibit the activation of plasminogen by urokinase.

α_2-macroglobulin (Immediate reacting antiplasmin) has a mol.wt. of 725,000, it is thermostable and reacts irreversibly with plasmin and other serine proteases (Jones et al.1972,Barrett and Starkey 1973). Frénoy et al.(1972) showed that the molecule is built up of identical subunits and linked together by disulphide bonds. Ganrot and Nilehn (1967) found that 1 mol.of α_2-macroglobulin binds 1 mol.of plasmin. They also found that plasmin and thrombin compete for the same site of the molecule. Recently, it was reported by Schreiber et al. (1973) that 1 mol. of α_2-macroglobulin inhibits two molecules of plasmin. α_2-macroglobulin has been shown to bind and inhibit essentially all four classes of endopeptidases (Barret and Starkey 1973,Heimburger 1974). In the formation of an enzyme-α_2-macroglobulin complex, hydrolysis of a peptide linkage occurs and through cleavage of this peptide

bond a conformational change is triggered in the α_2-macroglobulin-molecule. As a result the proteinase is irreversibly bound without involvement of covalent linkages. Only molecules of definite size may have excess to the entrapped proteinase (Heimburger 1974). The α_2-macroglobulin-enzyme complexes are very rapidly cleared from the circulation (Ohlsson and Olsson 1975).

α_1-antitrypsin is a glycoprotein enzyme inhibitor found in the serum α-1-globulins. The mol.wt. has been found to be about 55,000 (Schultze et al.1955,Jeppsson and Ericsson1975). α_1-antitrypsin is also a protease inhibitor with a specific ability to neutralise the elastolytic proteinases of leukocytes (Ohlsson and Olsson 1974, Heimburger 1974), enzymes that are mediators of inflammation and attacks components in connective tissue, collagen and elastin but probably also the proteoglycans in the connective tissue and basement membranes (Ohlsson and Olsson 1975). The earlier proposed inhibition of trypsin and plasmin of α_1-antitrypsin (Cohen 1973) is probably a test tube phenomena and its biological function is concerned rather with other proteases (Jeppsson and Laurell 1974). Granulocyte proteinases, which are protective factors against infectious agents inducing inflammation, may become injurious agents by digesting tissue structures (Steinbuch and Audran 1974,Cutz et al.1974). The circulating protease inhibitors are probably of importance in limiting the tissue damaging effect of the granulocyte proteases. α_2-macroglobulin and α_1-antitrypsin vary independently of each other in pathological conditions (Miesch et al.1971). According to Nilēhn and Ganrot (1967), the α_2-macroglobulin is presumably the most important antiplasmin because of its extreme fall in concentration during thrombolytic therapy with streptokinase.

Fibrinogen and Fibrin stabilising factor (Factor XIII)

The fibrinogen molecule is a dimer, and is built up of two identical halves (Blombäck et al.1972). According to Gaffney and Dobos (1971) and McDonagh et al.(1972) each of these halves consists of 3 different peptide chains, A α (mol.wt.64,000), B β (mol.wt. 57,000) and γ (mol.wt.48,000). The complete fibrinogen molecule thus has a six chain structure with a mol.wt. of 340,000 (McDonagh et al.1972). The three chains are linked by disulphide bonds and the two halves of the molecule are held together by at least

three symmetrical disulphide bridges. 40% of the disulphide bridges are located in the hydrophilic N-terminal portion in the so-called disulphide knot (mol.wt.60,000, Blombäck et al. 1968). Three additional disulphide knots, which are hydrophobic, have recently been demonstrated by Gårdlund et al.(1972). This has, however, been questioned by Finlayson (1975).

The N-terminal part of the molecule contains the fibrinopeptides A and B, which are situated in the A_α- and B_β-chains, and are split off by the action of thrombin on the fibrinogen molecule (Bailey and Bettelheim 1955). Thrombin splits only arginyl-glycine bonds in the A^α- and B^β-chains (Blombäck 1971, Blombäck et al.1972) and releases N-terminal residues of glycine in the fibrinogen molecule (Kierulf and Abildgaard 1971). The fibrin monomers aggregate and become stable and under influence of factor XIII and Ca^{2+}, the fibrin clot form. This so-called cross-linking is a transamidase catalysed reaction with the formation of covalent bonds between the glutamyl residue in one fibrinogen molecule and the amine group of the lysine residue of another fibrinogen molecule (Lorand 1970).

Doolittle et al.(1972) have shown the formation of 6 mol. cross-links/mol.fibrin, four are contributed by theα -chains and two by the $^\gamma$-chains. Plasma factor XIII contains two forms of polypeptide chains(a and b) and each molecule consists of two a-chains and two b-chains. The mol.wts. of the a- and b-chains have been determined as 75,000 and 88,000, respectively (Schwarz et al.1972). The mol.wt. of plasma factor XIII was determined by the same research group as 320,000. A large amount of factor XIII has also been shown in the platelets (McDonagh et al.1969). Its mol.wt. is 150,000-200,000 and contains only two chains, both of a-type (Schwarz et al.1972).
It is assumed that the liver plays a major role in the biosynthesis of fibrinogen (Miller and John 1970). However, also extrahepatic formation of fibrinogen has been assumed, since the plasma fibrinogen level is often normal in various types of liver damage (Ansari and Silvis 1972).

Fibrin/fibrinogen degradation products (FDP)

Degradation of fibrinogen by plasmin results in the formation of first high molecular weight degradation products (HMWDP), designated X (mol.wt.240,000) and Y-fragments (mol.wt.155,000)

(Marder 1971). The X-product is coagulable with thrombin, while the Y-product is not. Fragment X is a heterogenous population with different mol.wts. (248,000 to 252,000) because of different degrees of degradation of the A_α- and $B\beta$-chains (Pizzo et al.1972). A-, B- and C-peptides are released at the same time as the X-product appear (Marder and Shulman 1969). These are peptides split off the A_α-chain and do not possess the antigenic properties of fibrinogen (Pizzo et al.1972). Fragment Y contains the disulphide knot of the fibrinogen molecule and partially degraded A_α-, B_β- and γ-chains. Further degradation of the Y-fragment results in the formation of a D- and a E-fragment, called the end degradation products, which have antigenic determinants in common with fibrinogen (Nussenzweig et al.1961). The mol.wt. of the D-fragment varies between 45,000 and 160,000 (Pizzo et al.1972), according to the extent of degradation of the γ-chain. It has been shown that the D-fragment derived from cross-linked fibrin differs from that derived from fibrinogen in that it contains a dimer of the degraded γ-chain including the cross-linking site of the molecule and two each of the degraded α-and β-chains (Pizzo et al.1972).

The D-fragment, recently studied by several authors, is very heterogenous (Gaffney and Brasher 1973, Furlan and Beck 1973). Mosesson et al.(1973) even suggested that a given fibrinogen molecule gives rise to only one type of D-product. E-fragment (mol.wt.41,000) consists of the disulphide knot of the fibrinogen molecule (Wallén 1971, Pizzo et al.1972, Gaffney and Brasher 1973). The E-fragment derived from fibrinogen is identical with that derived from fibrin (Taylor et al.1972). Edgington and Plow (1972) have contended that fragments D and E combine in a 1:1 molar ratio and that one D:E is derived from each half of the fibrinogen molecule. They also produced evidence of a neoantigen of fibrinogen situated in the D-fragment exposed by the cleavage process (Plow and Edgington 1973,-75). They also found neoantigens specific for fibrinogen D-fragment or fibrin D-fragment, which makes it possible to separate fragment D derived from fibrinogen and that derived from fibrin. According to Nilehn (1967) 50% of HMWDP and 10% of D-and E-fragments are retained in the clot. Ly and Arnesen (1973/74) demonstrated that at pH 7.8, 50-60% of early FDP were incorporated in clots, while late FDP were incor-

porated to only a minor extent. When the pH was lowered to 6.3, the incorporation of early FDP was markedly reduced.

The behaviour of human fragment D in rabbits has been studied by Hayne and Sherman (1973). They found two components in plasma clearance of ^{125}I fragment D *viz* 66.0±6.0% was cleared with a half-time of 0.9±2 hrs and 32.3±5.3% with a half-time of 3.6±0.3 hrs. In nephrectomised animals the first component of plasma clearance was normal, while the second was prolonged, indicating that the kidneys are important in the catabolism of one portion of fragment-D.

In experimental studies, Rayner at al.(1969) demonstrated that intravenously infused D- and E-fragments are cleared from the blood through the kidneys while HMWDP are not.

Collection of blood

Plasma was prepared from blood collected into siliconised centrifuge tubes containing 1 volume of 3.8% trisodium citrate solution x 2 H_2O. The blood samples were immediately centrifugated at 2,000 g for 20 minutes and plasma was withdrawn by means of siliconised pipettes and distributed among plastic tubes. The plasma were stored at -70°C until used.

Serum was obtained from blood collected in glass tubes. After standing for 2 hrs at room temperature, the tubes were centrifugated at 2,000 g for 20 minutes. The serum was withdrawn by means of siliconised pipettes and dispended in glass tubes. The samples were stored at -20°C.

Collection of urine for FDP determination

A) Morning urine (10 ml) was collected without addition of any inhibitor of fibrinolysis. The samples were stored at -70°C until tested. B) The 24 hr urine volumes were collected without addition of any EACA. The urine samples were concentrated by ultrafiltration with a membrane that retained proteins with a mol.wt. of more than 12,000 (Diaflo Ultrafilter Membrane, Amicon Corporation,Massachussets) and by dialysing against polyethylenglycol until the protein concentration was about 70 g/l (Biuret method).

Platelet counts were made by the method of Björkman (1959). Normal range: 155,000-365,000/mm^3.

Bleeding time (method of Ivy and Duke) was performed according to Nilsson et al.(1963). Normal range: 6-12 min. and 1-5 min., respectively.

Platelet adhesiveness in whole blood of Hellem (1960), as modified by Cronberg (1968). Normal range: 17-35%.

Coagulation time, recalcification time, prothrombin consumption, one-stage prothrombin time, P & P were performed according to methods described by Nilsson et al.(1961).

APTT was performed as described by Nilsson (1974).

Factor V was determined according to Wolf (1953).Normal range: 80-120%.

Fibrinogen. Citrated blood was collected with EACA and the sample assayed with a synerese method according to Nilsson and Olow (1962). Normal range: 0.20-0.40 g/100 ml.

Factor VIII activity was assayed on platelet-rich hemophilia A plasma (factor VIII activity < 1%) in a recalcification system as described by Nilsson et al.(1959). The hemophilia A test substrate was obtained with the silicone technique and centrifugated at 185 g for 20 minutes. It was checked that the hemophilic plasma contained at least 200,000 platelets/cu.mm. The plasma was stored at -60°C for at most one month before use. The plasma to be tested was prepared by immediate centrifugation of the citrated blood for 20 minutes at 2,400 g at room temperature and was stored at -60°C until examined. The 100 % standard plasma was a mixture of plasma from 20 normal donors. The plasma to be tested was assayed at dilutions of 1:50, 1:100 and 1:200 in 0.9% saline. The method has, after 15 years experience at our laboratory, been shown to be very reliable in the hands of experienced technicians. The reproducibility of the values is very good, which has been shown by comparision with international standards (Miller-Andersson 1974). Normal range:60-160%.

Factor VIII related antigen was quantitated with Laurell's electroimmunoassay (1972), as described by Holmberg et al.(1973). Heterologous antiserum to factor VIII was prepared by repeated injections of 1 ml purified factor VIII solution emulsified with Freunds complete adjuvant in rabbits. 2 weeks after the booster dose was given, the rabbits were bled. The blood was allowed at least 2 hours to clot and the serum was separated. off. The quantitative determination of factor VIII antigen was performed with Laurells electroimmunoassay (1972). Plasma was tested undiluted and in dilutions 1:2 and 1:4. The electrophoresis was run in 1% agarose for 16 hours at 5 V per cm. The reproducibility of the method was good. Normal values: 60-160%.

Ethanol gelation test according to Godal et al.(1971).

The fibrinolytic activity of plasma and resuspended euglobulin precipitate was measured on unheated bovine fibrin plates, as described by Nilsson and Olow (1962). Normal for plasma: 24 ± 35 mm^2 and for resuspended euglobulin precipitate: 98 ± 54 mm^2.

Euglobulin clot lysis time Nilsson and Olow (1962). Normal:>100 min

Plasminogen was determined with an immunochemical method described by Ganrot and Nilehn (1968) as modified by Ekelund et al.(1970). Normal range: 70-130%.

Fibrin/fibrinogen degradation products (FDP)

Blood samples were collected with epsilon aminocaproic acid (EACA) to prevent fibrinolysis in vitro (5-10 mg/ml blood) and thrombin (3 N.I.H. units/ml blood) to get a complete coagulation. Serum was prepared according to Paraskevas et al.(1962). Urine samples (10 ml) or 24 hr urine samples were collected without addition of EACA as mentioned above.

FDP in serum and in urine were determined according to the immunochemical method of Nilêhn (1967), using a specific antiserum against the D-product in the rocket method of Laurell (1966). The serum (dil.1/1) and urine samples (dil.1/1), unconcentrated and concentrated, were applied to agarose plates (1% agarose) containing the anti-D serum and on high voltage electrophoresis (20 v per cm) the antigen is forced into the gel. Precipitation peaks form, and their heights are proportional to the amount of antigen in the sample. High molecular weight degradation products (HMWDP) were used as a standard. No FDP could be demonstrated in serum or urine from 200 healthy controls. No FDP was found in concentrated urines from 19 controls (paper IV).

Urinary FDP were also determined by radioimmunoassay (paper VII). In this method the HMWDP were degraded further to D-fragments by addition of streptokinase and plasminogen to the urine samples, and the D-products were afterwards determined with a specific anti-D serum.

Typing of FDP were performed by the method of Bouma et al.(1971), based on Laurell's rocket method (1966). Urine (dil.1/1) or serum (dil.1/1) was applied to separate agarose gel plates containing antisera against human fibrinogen, D-product and E-product, respectively. The antisera were prepared according to Nilêhn (1967). On high voltage electrophoresis the antigen is forced into the gel. Precipitation peaks form, the heights of which are proportional to the amount of antigen in the samples. Fibrinogen is used as a standard for all the plates. A mixture of different types of FDP forms two or three peaks because of differences in molecular weight, charge and antigenic determinants. By demonstrating single or double peaks against the different antisera it is possible to decide the type or types of FDP and their concentrations in relation to fibrinogen. Addition of substances identical with that present in the urine results in an increase in the height of the peak. If the substances is not identical, the result is a double peak.

Inhibitors of plasminogen activation in serum were determined according to a clot lysis method described by Paraskevas et al. (1962). Normal range: 60-140%.

α_2-macroglobulin were determined with an esterolytic method described by Ganrot (1966). Normal range: 80-120%.

Urokinase activity in urine was determined in unconcentrated urine with Andersson's clot method (1962). In 10 control persons values above 8 Ploug units/ml were obtained (paper IV).

Fibrin slide technique. The fibrinolytic activity of renal tissue was assessed histochemically according to Pandolfi (1972). Frozen sections, 4-6 µm thick, were covered with a mixture of bovine plasminogen-rich fibrinogen and thrombin. A 70 µm fibrin film was obtained. Four slides were prepared for each specimen. The slides were incubated in a moist chamber at 37°C for 0, 10, 20 and 30 minutes. The fibrin film was digested at the sites of fibrinolytic activity. The fibrinolytic activity was estimated from the focal lysis time, that is the minimal incubation time required to obtain well-defined areas of lysis (Kwaan and Astrup 1963).

Serum creatinine was measured with a Technichon method used routinely at the Department of Clin.Chem.Lab. Normal range: ♂ 0.8-1.4 mg/100 ml, ♀ 0.6-1.3 mg/100 ml.

Albumine in urine was determined immunologically with brom-cresolgreen method according to Doumas et al.(1971) and low values were corrected by electroimmunoassay (Laurell 1972). Limit value: <25 mg/l.

24 hour endogenous creatinine clearance, Addis quantitative sediment were determined routinely with standard techniques used at the Clin.Chem.Lab. Normal range: 96-148 ml/min/1.73 m^2.

Orosomucoid, α_1-antitrypsin, ceruloplasmin, C3 and C4 were estimated with the electroimmunoassay of Laurell (1972) at the Dept. of Clin.Chem. Normal range: 50-138%, 70-130%, 0.25-0.50 g/l, 70-130%, and 65-170%, respectively.

Haptoglobin was determined according to Tarukoski (1966). Normal range: 0.3-1.8 g/l.

^{51}Cr-EDTA clearance was performed according to Bröchner-Mortensen (1969). Age-variation.

Renal biopsy was performed with a disposable renal biopsy needle according to Hamburger. Direct fluoroscopy was used to locate the right kidney. For light microscopy the biopsy specimen of the kidney was fixed in neutral formalin, embedded in paraffin and sectioned 3 µm thick. Staining with haematoxylin and eosin, PAS, Weigerts staining for elastin and periodic silver method, PTAH and alk.Kongored.
The kidney specimens from the patients with glomerulonephritis were studied by the same two pathologists during the whole time. For this study all of the specimens were reevaluated by one of the pathologists. The slides were studied without knowledge of the further course of the disease. Very few changes in diagnosis were made during the reevaluation.

Immunofluorescence microscopy was performed at the Clin.Chem. Lab. Kommunehospitalet, Copenhagen with a technique including a direct method technique with FITC conjugated antifibrinogen.

Methods using immunofluorescence- and electronmicroscopy have demonstrated large amounts of fibrin/fibrinogen related material in the kidneys in the hemolytic uremic syndrome (HUS) and in thrombotic thrombocytopenic purpura (TTP) (Lieberman et al.1966,Habib et al.1969, King et al.1969,Brain 1969,Gervais et al.1971,Lieberman 1972,Kincaid Smith 1972,Franklin et al.1972, Jaffe et al.1973,Brown et al.1973).

Also in certain types of glomerulonephritis, especially the proliferative such deposits occur in the capillaries, in the walls of arteries, subendothelially along the basement membrane, and intramembranously, but above all in crescents of glomeruli (Vassalli et al.1964,Paronetto and Koffler 1965,Humair et al.1969, Cameron 1970,Kincaid Smith et al.1970,Vassalli and McCluskey 1971, Clarkson et al.1971,Chirawong et al.1971,Kurtz et al.1972, Davison et al.1973).

Fibrin/fibrinogen related material occur also immediately after renal transplantation in the graft and particularly in large amounts in acute rejection (Kincaid Smith 1967, Starzl et al.1968,Salaman 1972,Claes et al.1973).

Intraglomerular fibrin deposits can also be detected in experimental glomerulonephritis (Vassalli and McCluskey 1964). Besides the fibrin deposits in this condition characteristic histologic lesions of glomerulonephritis develop in situations without involvement of immunologic mechanisms. Treatment with warfarin and heparin prevents the glomerular sclerosis.

The origin of the fibrin deposits in the above-mentioned conditions have been discussed.

In HUS the fibrin thrombi are confined to the renal microvasculature, but in TTP the histological picture is characterised by widely disseminated thrombi in small blood vessels with associated endothelial hyperplasia in multiple organs. HUS has been described as the result of an immunologic process, i.e. circulating antigen-antibody complexes are thought to give rise to an immune complex glomerulonephritis, with secondary local intravascular coagulation and deposition of fibrin in the kidneys

(Gianantonio et al.1964,Fluge et al.1967,Gervais et al.1971, Katz et al.1971). Glomerular deposits of IgM and C3 have recently been demonstrated by McCoy et al.(1974) with immunofluorescence microscopy. But most authors have not found any depositions of gamma globulins or complement (Habib 1969,Gervais et al. 1971,Lieberman 1972). But, as a rule, only fibrin/fibrinogen related material can be demonstrated (Lieberman et al.1966, Vitsky et al.1969, Lieberman 1972,Jaffe et al.1973,Powell and Ekert 1974).

It has also been claimed that disseminated intravascular coagulation occurs in HUS and TTP (McKay et al.1967,Monnens et al.1967, Piel and Phibbs 1966,Stiehm et al.1969,Gilchrist et al.1969, Brain 1969,Uttley 1970,Vitacco et al.1973, van Wieringen et al. 1974). Brain et al.(1967) found, for example, accelerated ^{131}I-fibrinogen turnover in HUS. Detailed sequential studies of the coagulation factors in these conditions are, however, limited. Most studies of the coagulation and fibrinolysis in these conditions have revealed a low platelet count, normal or increased amounts of fibrinogen, high factor VIII content, high or normal values for factor V, prothrombin and plasminogen, negative ethanol gelation test and low fibrinolytic activity. FDP are generally demonstrable in the serum, but still more often in the urine (Avalos et al.1970,Katz et al.1971,Monnens et al.1972, Lieberman 1972,Kaplan et al.1971,Jaffe et al.1973,van Wieringen et al.1974). These findings weigh against the occurrence of disseminated intravascular coagulation.
Using ^{51}Cr-labelling, George et al.(1974) demonstrated a shortened platelet survival, which they concluded to be secondary to primary intrarenal endothelial cell damage in HUS.

HUS and TTP can develop secondarily in malignant hypertension, pregnancy, pre-eclamptic toxemia and postpartum renal failure (Brain et al.1962,Vencatchalam et al.1968,Linton et al.1969, Kincaid Smith 1972,Ekberg et al.1974). The primary vessel damage and the disturbances of the microcirculation in these conditions have been thought to induce intravascular coagulation and microangiopathic hemolytic anemia.

Glomerulonephritis is an immunologic disease, in which several mechanisms can cause deposition of fibrin (Dixon 1968).

Two immunologic mechanisms are active in glomerulonephritis (Dixon 1967, -71): a) action of antibodies to some antigenic component of the glomerular basement membrane, and b) formation of circulating antigen-antibody complexes, in which the antigen is not necessarily of glomerular origin. These complexes are soluble and accumulate in glomeruli. The complement system can be activated in two different ways: the classic pathway, which is activated when antibody combines with antigen. The second is the alternative pathway of properdin activation (Götze and Müller-Eberhard 1971). Both result in application and attraction of polymorphonuclear leucocytes followed by release of enzymes causing denudation of the basement membrane (Cochrane 1971, Henson 1971). As mentioned earlier, the platelets adhere to the denuded collagen fibers, and initiate thereby the primary hemostasis resulting in fibrin deposits (Jamieson et al.1971, Jaffe et al.1974,Kerr 1975).
That activation of the complement system can also initiate blood coagulation has been demonstrated by Zimmerman and Müller-Eberhard (1971), as summarised by Merrill (1974).

According to several authors, the deposition of fibrin in glomerulonephritis is the result of disseminated intravascular coagulation (McKay 1965,Hardaway 1966,Wardle et al.1968,Haanen et al.1971). The literature contains no studies of the coagulation in the early stages of glomerulonephritis, but such investigations have been performed in later stages of renal disease with uremia. Thus, in acute and chronic renal insufficiency in various renal diseases Larsson et al.(1971) found normal platelet counts, high values for factor VIII and fibrinogen, normal or increased factor V and P & P and plasminogen, increased values for inhibitors of plasminogen activation and low values for α_2-macroglobulin, low spontaneous fibrinolytic activity in the blood, negative ethanol gelation test and high content of FDP in serum and urine. These changes were similar to those seen in processes combined with local deposition of fibrin in the kidneys and argue against the occurrence of disseminated intravascular coagulation.

In renal transplantation fibrin deposits appear in the graft

soon after the revascularisation (Claes et al.1973). Even in uneventful renal transplantation there is an accumulation of fibrin, though less massive, than during an acute rejection episode (Claes et al.1973).
Ljungqvist et al.(1969), who studied coagulation and fibrinolytic parameters for 2-3 weeks after successful transplantation in 8 patients, found the parameters to vary in largely the same way as after other major operations (Olow 1963). But fibrin deposits occur in the renal graft also immediately after autotransplantation, and are presumably caused by the ischaemic damage.

In acute rejection an antigen-antibody reaction occurs and results in injury to the vessel endothelium by the inflammatory reaction, with secondary aggregation of platelets in the graft (Bettex-Galland et al.1963,Dempster 1953,Hume et al.1955,Starzl et al.1968). This aggregation leads to release of substances that initiate the coagulation process with consequent formation of fibrin thrombi.

In rejection the turn-over of fibrinogen and platelets is increased (Claes et al.1973). Starzl et al.(1968,-70) found low values for the various coagulation factors in rejection and interpreted it as caused by disseminated intravascular coagulation. Colman et al.(1969) found no evidence of consumption of the coagulation factors during rejection episodes. Wardle et al.(1975) found an increased catabolism of fibrinogen in acute and chronic rejection, an increase not necessarily due to activation of the coagulation system.

Digestion of the glomerular fibrin/fibrinogen deposits in the above mentioned conditions requires the presence of at least locally fibrinolytic capacity. As mentioned above, 2 types of fibrinolytic activators occur in the kidney, viz tissue plasminogen activator and urokinase.

With Todd's histochemical method it is possible to demonstrate the tissue activator in the medullary and in the large intrarenal blood vessels (Pandolfi et al.1971), but not in the renal cortex. Humair et al.(1969) observed, however, that fibrinolytic activity could be elicited in the glomeruli of the rat kidney by inducing coagulation and precipitation of fibrin in the kidney. Fibrin has also recently been found to enhance fibrinolytic activity in

in vitro cultures of kidney (Bernik 1973). Pandolfi et al. (1971) also showed that the glomeruli became fibrinolytically active in chronic inflammation and in rejection of renal grafts.

The urokinase has also been shown to disappear already in an early phase of glomerulonephritis and after renal transplantation (Vreeken et al.1966,Carlsson et al.1970), and must therefore be of minor importance in the dissolution of the glomerular fibrin deposits (Åstedt and Pandolfi 1971).

It is, however, possible that the collagenases and elastases released from the accumulated granulocytes in glomerulonephritis also are of importance for the degradation of fibrin/fibrinogen, since they have been found to possess fibrinolytic activity (Ohlsson and Olsson 1975).
The fibrinolytic activity in the blood is low in renal diseases (Larsson et al.1971,Ito et al.1972). It is now generally accepted that this is due to an increased concentration of inhibitors of plasminogen activation (McNicol 1965,Wardle et al.1970,Larsson et al.1971).

FDP in serum and urine are found predominantly in the acute progressive glomerular diseases when renal function is impaired (Wardle and Taylor 1968,Stiehm and Trygstad 1969,Larsson et al. 1971,Hedner and Nilsson 1971,Ekert et al.1972,Briggs et al.1972). Urinary FDP can also be found in certain types of nephritis, especially proliferative glomerulonephritis even when renal function is normal (Naish et al.1970,Clarkson et al.1971,Preston et al.1971,Ekert et al.1972). In membranous glomerulonephritis, however, the concentration of urinary FDP is, as a rule, substantially lower, and occur less often (Clarkson et al.1971). The concentration of urinary FDP varies closely with the amount of fibrin deposits in the kidney in different types of glomerulonephritis (Stiehm and Trygstad 1969,Chirawong et al.1971,Clarkson et al.1971).
The FDP concentration in urine has also been claimed to vary with the activity of the disease (Braun and Merrill 1968,Stiehm and Trygstad 1969,Larsson et al.1971,Clarkson et al.1971).

The urinary FDP have been typed by Vermylen et al.(1972) and Clarkson et al.(1971) in glomerulonephritis and both found predominantly low molecular weight fragments in the proliferative types of glomerulonephritis. Clarkson et al.(1971) distinguished between proliferative forms of glomerulonephritis, in which they found low molecular weight fragments, and membranous glomerulonephritis, in which they found high molecular weight fragments in only low concentration. Bouma et al.(1971) found high molecular fragments in patients with acute and chronic uremia.
Urinary FDP have always been found during the first 2 weeks after renal transplantation and are ascribed to the ischaemic damage of the kidney (Braun and Merrill 1968,Antoine et al.1969,Clarkson et al.1970,Grob et al.1971). The appearance and later increase of FDP is an early sign of rejection (Antoine et al.1969,Carlsson et al.1970,Clarkson et al.1970,Grob et al.1971,Shah et al.1972, Naish et al.1973). Typing of urinary FDP after renal transplantation has sometimes revealed low molecular weight fragments (Braun and Merrill 1968,Zühlke and Herman 1970,Haanen et al.1971) and sometimes both low and/or high molecular weight fragments (Antoine et al.1969,Grob et al.1971,Bouma et al.1971).

PRESENT INVESTIGATION

The aims were

1. to elucidate the mechanism of the development of the fibrin deposits in glomerulonephritis and hemolytic uremic syndrome, by following the variation of various coagulation and fibrinolytic factors during the course of the disease (Papers I,II,III and VI);

2. to assess the prognostic value of determining factor VIII in glomerulonephritis (Papers I and II);

3. to find out whether any association exists between the glomerular fibrin deposits and the presence of glomerular fibrinolytic activity, on the one hand, and the urinary FDP, on the other (Paper III);

4. to estimate the value of determining urinary FDP in glomerulonephritis, in renal transplantation and in hemolytic uremic syndrome (Papers I,II,IV,V and VI); and

5. to work out a more sensitive and precise method for determining urinary FDP (Paper VII).

I. COAGULATION- AND FIBRINOLYTIC PATTERN IN EARLY DETECTED GLOMERULONEPHRITIS

The material consisted of 116 patients admitted to hospital for the first time for investigation of proteinuria and/or hematuria and suspected glomerulonephritis. The diagnosis was verified by percutaneous renal biopsy and the patients were accordingly grouped histologically. Renal function, as judged from 3 consecutive determinations of the serum creatinine and 24 hr endogenous creatinine clearance was normal in 110 of the patients, but was moderately impaired in the remaining 6. Renal damage was also judged from the severity of albuminuria and hematuria.

The patients were followed up regularly for up to 4 years. Follow-up included examination of renal function as well as of the coagulation- and fibrinolytic systems.

The patients were divided into 4 groups according to the state of renal function and damage at the end of follow-up.

Group I (27 patients): serum creatinine and glomerular filtration rate were normal and no albuminuria and hematuria at the end of follow-up. Group II (42 patients): serum creatinine remained unchanged or increased by at most 0.2 mg/100 ml, but all still had albuminuria and/or hematuria during follow-up. Group III (31 patients): serum creatinine increased by 0.3-1.0 mg/100 ml during follow-up. Group IV (16 patients): serum creatinine was increased by more than 1.0 mg/100 ml at the end of follow-up.

The results of the examination of the coagulation- and fibrinolytic systems in the various groups are given below.
Ivy bleeding time was normal in group I, II and III on admission and during follow-up, but prolonged and remained so in group IV.

Platelet counts were normal in all of the 116 patients.

Platelet adhesiveness was normal initially in all of the patients, but decreased in those patients who developed impairment of renal function.
Factor VIII activity was normal initially in all of those patients who recovered (group I), while the mean value was close to the upper limit of the normal range in those who still had albuminuria and/or hematuria during follow-up (group II). In groups III and IV the factor VIII activity was initially abnormally high. The differences in initial mean values between group I and groups III and IV were highly significant.

The mean P & P level was normal in group I, II and III, but in group IV it was close to the upper limit of the normal range.

Factor V was normal in the four groups initially and during follow-up.

Fibrinogen: the mean value in group I fell within the limits of the normal range, but in half of the patients the level was abnormally high. In the other groups the mean value was high from the beginning and remained so in groups III and IV.

The fibrinolytic activity of the plasma was low in all of the patients in the 4 groups.

Inhibitors of plasminogen activation were increased in 6 of the patients in group I, but were normal in 21. The levels were normal in 8 of the patients in group IV but elevated in the remaining 8.

There was a significant difference in mean value between group I and groups III and IV, respectively.

α_2-macroglobulin was initially increased in half of the patients in group I, but normal by the end of follow-up. In the other groups the α_2-macroglobulin level was initially high and persisted so during follow-up in those patients in group IV who developed severe renal insufficiency.

	Group I	Group II	Group III	Group IV	
Ivy bleeding time (min)	11.8±4.0	12.1±4.1	11.0±3.5	14.6±7.1	8-12
Platelet adhesiveness (%)	17.3±5.0	21.2±3.0	23.1±9.4	17.8±7.2	17-33
Factor VIII (%)	111.6±24.1	151.7±57.6	209.9±61.1	229.1±79.1	60-160
P & P (%)	98.1±20.2	103.3±26.6	110.1±22.7	122.1±25.2	80-120
Fibrinogen (g/100 ml)	0.37±0.13	0.42±0.18	0.44±0.14	0.60±0.15	0.20-0.40
FDP/urine (mg/24 hr)	0.4	0.2	4.0	13.2	0
Urokinase inhibitors (%)	123.6±30.2	146.8±45.2	160.7±40.0	155.9±46.4	60-140
α_2-macroglobulin (%)	144.3±35.6	153.9±100.2	184.5±67.8	161.1±83.7	80-120

FDP in urine were found in 50 of the patients in group I and II, but disappeared during remissions. 26 patients in groups III and IV had initially high levels of urinary FDP, which persisted.

Acute phase reactants. No significant difference was found between the means of any of the groups.

II. FACTOR VIII AND GLOMERULONEPHRITIS

Material I: consisted of 70 patients of the 116 in paper I, admitted to hospital for investigation of suspected glomerulonephritis. The diagnosis was verified from percutaneous renal biopsy in all the cases. Renal function, as judged from 3 consecutive determinations of the serum creatinine and creatinine clearance, and in 26 of the patients from ^{51}Cr-EDTA clearance, were normal, except in a few patients in whom renal function was moderately reduced. These patients were followed up for up to 4 years and during which renal function was regularly examined. Factor VIII activity and factor VIII related antigen were investigated on admission of the patients to hospital and at regular intervals during follow-up.

The patients were divided into 4 groups in the same way as in paper I.

Material II: We studied also the factor VIII activity and related antigen only on admission in 15 patients with recently discovered glomerulonephritis and impaired renal function, as judged from ^{51}Cr-EDTA clearance.

The results of the mean initial values of the factor VIII activity and the factor VIII related antigen in the patients are given in the Table.

	Group I (15 pat)	Group II (25 pat)	Group III (17 pat)	Group IV (13 pat)	Material II (15 pat)
Factor VIII activity (60-160%)	109.5±28.5	137.9±45.5	206.9±42.6	231.4±59.9	211.0±46.6
Factor VIII related antigen (60-160%)	105.5±34.8	155.2±60.9	237.4±71.2	305.2±83.7	245.5±46.5

The differences in mean value between group I and groups III and IV respectively were highly significant ($p < 0.001$).
The factor VIII level did not vary with the degree of albuminuria.
The factor VIII level was initially increased in all of the patients with impaired renal function, and in those patients who developed persistent renal damage during follow-up (groups III and IV).

III. ON THE ORIGIN OF FIBRIN/FIBRINOGEN DEGRADATION PRODUCTS IN GLOMERULONEPHRITIS

The material consisted of 28 patients, admitted to hospital because of proteinuria and/or hematuria and suspected glomerulonephritis, but with normal renal function. The diagnosis was verified by percutaneous renal biopsy. Renal fibrinolytic activity were studied with the fibrin slide technique according to Pandolfi (1971). The tissue was studied for presence of fibrin/fibrinogen related material by the immunofluorescence technique.

FDP in serum and in urine were determined as was the fibrinolytic activity and fibrinolytic inhibitors in blood.

Fibrinolytic activity was found in 25 of the 27 specimens of the renal cortex in patients with glomerular disease, while no fibrinolytic activity was found in specimens of the cortex of 8 healthy kidneys. The activity was confined to the glomeruli and small cortical vessels in glomerulonephritis.

In 14 of the patients immunofluorescence studies revealed fibrin/fibrinogen related material in the glomeruli. In all these 14 patients their renal cortex showed fibrinolytic activity.

No fibrin deposits were found in 9 of the patients who had fibrinolytic activity in the glomeruli.

Two patients had neither fibrin deposits nor glomerular fibrinolytic activity. Two had fibrinolytic activity, but in these 2 cases the kidneys were not examined with the immunofluorescence method.

FDP were demonstrated in the urine from 14 patients and 12 of them had fibrin deposits in the glomeruli.

There was a strongly significant correlation between the fibrin and the fibrinolytic activity, and also between the occurrence of fibrin and urinary FDP.

The spontaneous fibrinolytic activity in the blood was low in all except 3, and the inhibitors of fibrinolysis were increased in all those examined except 8.

Only one of the patients had FDP in the serum.

	FDP +	FDP -
Fibrin +	12	2
Fibrin -	2	10

Association between Urinary FDP and Glomerular Fibrin.

IV. URINARY FIBRIN/FIBRINOGEN DEGRADATION PRODUCTS (FDP) AND GLOMERULONEPHRITIS.

24 patients with different types of glomerulonephritis, classified according to Brun (personal communication), in whom renal function were normal, as judged from serum creatinine and 24 hr endogenous creatinine clearance, were studied for urinary FDP, determined according to the immunochemical method of Laurell, as described by Nilēhn (1967). Urinary FDP were determined and typed before and after concentration of the urine.
No FDP were found in 19 healthy controls after concentration of a 24 hr volume of urine.

In none of the patients could FDP be demonstrated in serum. Only one of the patients had FDP in unconcentrated urine. However, after concentration of 24 hr samples, FDP were found in 20 of the 24 patients (30-1650 µg/24 hr).

Urinary FDP were typed in 12 of the patients with the various types of proliferative glomerulonephritis and were found to be of high molecular weight type. One of the patients, however, had D and E products. That patient had normal urokinase activity in the urine (11 Ploug units/ml), while the other patients had low activity.

In none of the patients was plasminogen or α_2-macroglobulin found

in the urine.
The proteinuria was found to be of selective glomerular type in all these patients.

4 patients with membranous glomerulonephritis were studied. 2 of them had FDP in concentrated urine (30 and 60 µg/24 hr), which were found to be of high molecular weight type.

The excretion of the urinary protein was 2.1-5.2 g/l, and in all these patients it was of unselective type.
None of these patients had α_2-macroglobulin or plasminogen in the urine.
Urokinase activity were low in all 4.

V. DETERMINATION OF FIBRIN/FIBRINOGEN DEGRADATION PRODUCTS IN URINE AFTER RENAL TRANSPLANTATION

The material consisted of 29 patients who had received kidneys from cadavers. The postoperative period ranged from 1 month to 4 years. 19 of the patients developed no signs of rejection during follow-up, while 10 had one or more acute episodes of rejection.

FDP were determined every day in the serum and in unconcentrated urine during the first postoperative weeks and afterwards at the outpatient department every third week. When FDP were no longer demonstrable in unconcentrated urine, 24 hour samples were concentrated 2-500 times with an ultrafilter that retained proteins with a molecular weight of more than 12,000.

I. Patients without signs of rejection (19 patients)
Early postoperative course. Urinary FDP could be demonstrated in unconcentrated urine in all of the 19 patients up to 25 days after the transplantation (0.6-65 mg/24 hrs) and for 2-3 weeks in serum (40-50 µg/ml).
Later course. FDP could afterwards be found in the concentrated 24 hr urine samples up to 12 weeks postoperatively (1.5-20 mg/24 hrs). After 12 weeks FDP could no longer be found in the concentrated urine in the patients with an uneventful course.When FDP could persistently be demonstrated more than 12 weeks postoperatively, it indicated some complication such as glome-

rulonephritis in the transplant or chronic rejection. Persistence of urinary FDP after renal transplantation for more than 12 weeks after the operation thus seems to indicate a bad, or at least less successful prognosis for the graft.
Typing of FDP in 10 of the patients revealed high molecular weight degradation products (HMWDP).

II. Patients with acute rejection (10 patients)
In acute rejection episodes FDP were demonstrated in very high concentrations (15-220 mg/24 hrs) in the unconcentrated urine. In 2 of the patients the FDP increase in the urine was the first sign of rejection. In 8 of the patients, however, other signs of rejection, such as decreasing sodium excretion or urinary output, increasing proteinuria etc. appeared on the same day. The decrease in FDP in unconcentrated urine was always the first sign of successful treatment of threatining rejection in those 7 patients where the rejection was reversible. FDP in the concentrated urine could be demonstrated in 6 of these patients for 4-12 weeks after the rejection episodes.

Typing of FDP in 6 of the patients during rejection showed invariably HMWDP.

Urokinase activity was less than 1 Ploug unit/ml in all of the 29 patients.

VI. COAGULATION STUDIES IN HEMOLYTIC UREMIC SYNDROME (HUS) AND THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP).

The material consisted of 11 patients, including 5 who had previously always felt well and who had now fallen ill with the classical picture of HUS with thrombocytopenia, hemolytic anemia and renal injury (Group I). Six patients had a history of severe basic diseases such as malignant hypertension, SLE, or toxicosis of pregnancy, and they had developed the picture of a microangiopathic hemolytic anemia, thrombocytopenia and progression of renal injury (Group II).

All the patients had initially low platelet counts and high levels of factor VIII, especially when determined with the immunological

method. Labelling of the platelets with ^{51}Cr was done in 2 severe cases and showed a half-time of only 0.5-1 hour. They all had hemolytic anemia and renal injury of varying severity. At the first examination of the coagulation status, 10 of the patients had normal fibrinogen levels, normal or high values for factor V, P & P, plasminogen, low spontaneous fibrinolytic activity in the blood and negative ethanol gelation test. FDP were invariably found in large amounts, both in the serum and urine. One of the patients with post partum renal failure (case 11), however, had multiple coagulation defects with low values of P & P, factor V, fibrinogen and plasminogen already on admission.

All the cases in group I and 3 of the cases in group II were followed up almost daily with determinations of the various coagulation parameters. In only one (case 3) of the cases did the various coagulation factors fall substantially during follow-up on the second day after admission, which, however, successively returned to normal during continued treatment. In all the cases there was a good correlation between the clinical improvement, increase in platelet count and disappearance of FDP in the urine.(Fig.).

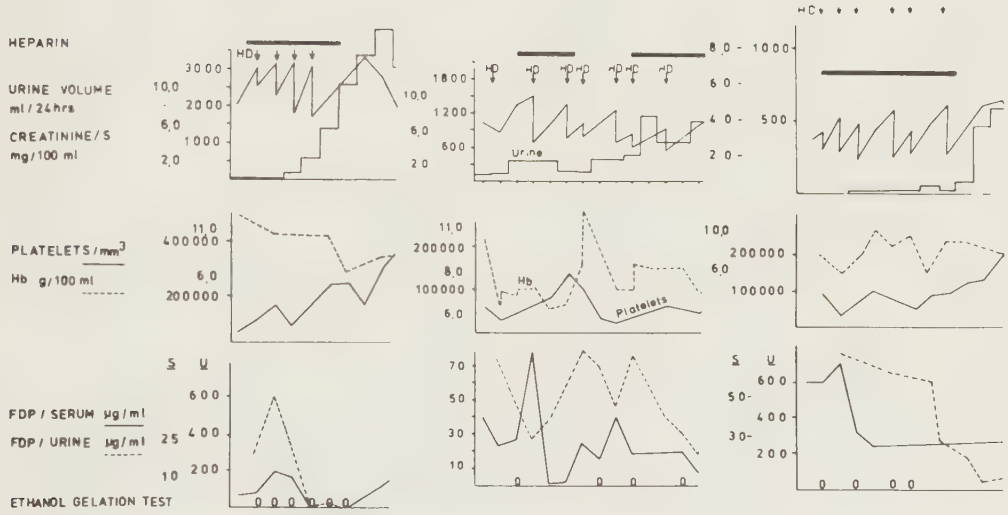
One of the patients in group I showed no impairment of renal function, but only proteinuria and hematuria. He received no treatment and made a complete recovery. Three patients in group I and 2 in group II were treated almost daily with hemodialysis for 3-4 hours, as soon as the diagnosis had been confirmed. The dialysis were continued until the production of urine had become normal and the serum creatinine had fallen spontaneously between the dialysis.

Low dose with heparin intermittently or continuously via the shunts (1,000-10,000 IU/24 hrs), was given from the very beginning in order to prevent clotting in the Scribner shunts in 3 cases in group I. If clinical manifestations intervened, which could suggest dissemination of the diseases with symptoms from various organs, or neurological symptoms (1 in group I and 2 in group II), the patients were given a continuous infusion of heparin in an endeavour to prolong APTT to twice its initial value.

Treatment with hemodialysis and heparin in the way described

above (3 in group I and 2 in group II) was followed by normalisation of the platelet count, increase in fibrinogen, disappearance of FDP in urine and improvement of renal function. These 5 patients recovered their renal function and no longer required hemodialysis. Heparin was given until the platelet count had become normal and the FDP/u had disappeared. None of the patients who were followed up had signs of a persisting renal injury or hypertension.

In one (No 5) of the cases, in which the patient did not receive this treatment, the picture developed into that of hemolytic uremic syndrome to a disseminated picture as in thrombotic thrombocytopenic purpura with renal cortical necrosis and the patient died with multiple dissemination of fibrin thrombi in all organs.



VII. DETERMINATION OF FIBRIN/FIBRINOGEN DEGRADATION PRODUCTS IN THE URINE BY RADIOIMMUNOASSAY

A highly sensible and specific radioimmunoassay for determining urinary FDP was worked out. In this method the HMWDP in the urine were completely degraded to low molecular weight fragments (D and E) by addition of streptokinase with plasminogen to the urine. D-products were afterwards determined with a specific antiserum.

D-fragment (Fg D) were prepared according to Nilêhn (1967). Antiserum against the D fragment (Anti-Fg D) was obtained by immunising rabbits with purified D fragment. For radioimmunoassay the antiserum was diluted to a final concentration of 1:250,000 and 200 µl of this solution bound approximately 65% of the Fg D-^{125}I added. Radioiodination of the D product was done with the lactoperoxidase method described by Thorell and Johansson (1971). The specific activity of Fg D was 40-50 µ Ci. In the radioimmunoassay procedure dilution was performed with assay buffer so that 200 µ l gave about 10,000 cpm. The radioiodination efficiency of this method was 80-90%. Standards were obtained by serial dilutions of purified Fg D solution (1.0 mg/ml) with buffer (250-1.9 ng/ml).

Pretreatment of urine samples. 0.1 mg of plasminogen and 100 IU of streptokinase were added to 1 ml morning urine. After 30 min. incubation in 37°C the HMWDP were completely degraded, which was checked on agarose plates after incubation of normal urine to which HMWDP had been added.

In the radioimmunoassay procedure 8 dilutions of standard Fg D and the treated urine samples were set up in triplicate. Labelled Fg D was added to each tube, followed by Fg D antiserum (diluted 1:250,000). Control tubes in triplicate, diluent blank and zero standard were also set up.

Antibody-bound and free peptide were separated by the second-antibody technique.

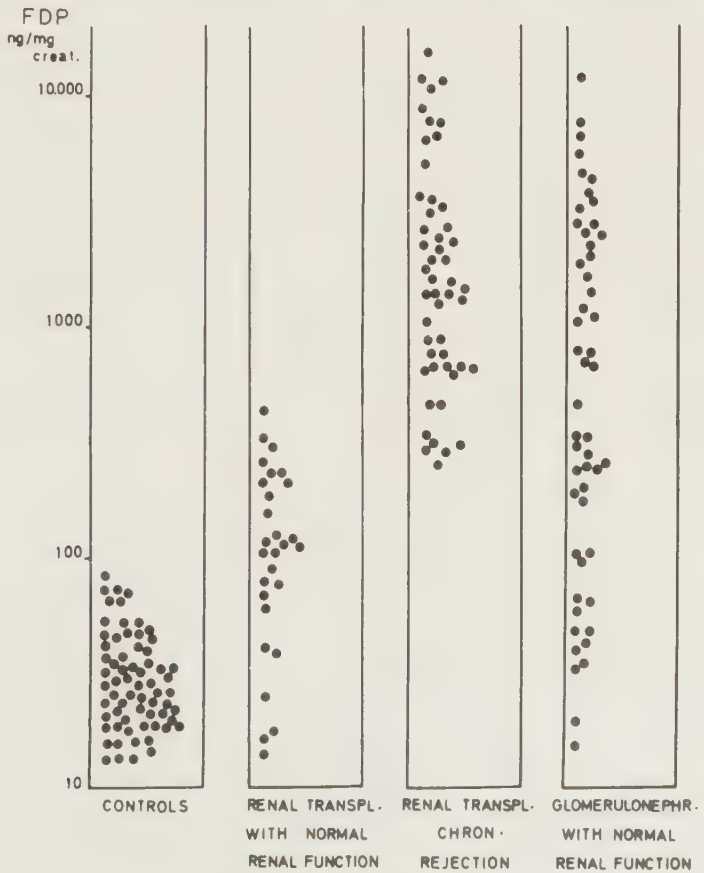
A dilution of the antiserum of 1:250,000 in the system was found to be optimal to cover the concentration range up to about 250 ng/ml. Further dilution was performed if the concentration was higher.

Other urinary substances did not interfere with the assay.

About 3 times more fibrinogen and about 8 times more HMWDP were required to get a 50% inhibition of binding, but Fg E gave no cross-reaction.

The lower limit of detection was estimated to about 5 ng/ml. Recovery experiments were run in several series, and the mean of recovery varied between 100-110%.

70 healthy persons had a urinary FDP concentration of 13-85 ng/mg creatinine. Fig. demonstrates the results of the FDP determinations in urine from 8 renal transplanted patients without signs of rejection and 6 with chronical rejection. It also demonstrates the urinary FDP determinations in 50 patients with chronical glomerulonephritis, but with normal renal function. Macroscopic hematuria was found to influence on the results obtained with this method, while microscopic hematuria had no effect.



Several investigations with immunofluorescence and electron-microscopy have shown that fibrin/fibrinogen related material sometimes occurs in glomeruli, especially in proliferative glomerulonephritis (Kincaid Smith 1970,Vassali and McCluskey 1971, Chirawong et al.1971). It is above all in so-called extracapillary glomerulonephritis that fibrin deposits in the crescents constitute a dominant component. But fibrin/fibrinogen related material has been demonstrated intracapillarily and subendothelially along the basement membranes also in other types of proliferative glomerulonephritis.

What is the origin of these fibrin deposits in glomerulonephritis?

It has been assumed that the fibrin deposits are the result of a disseminated intravascular coagulation precipitated by the primary disease (Wardle and Taylor 1968,Haanen et al.1971). It has, however, been questioned whether disseminated intravascular coagulation really is necessary for the formation of fibrin deposits, and it has been stressed that such deposits may occur as the result of local coagulation confined to the kidneys (Nilsson 1971,Kleinknecht et al.1973). Larsson et al.(1971) found in acute and chronic renal failure in glomerulonephritis a normal platelet count, normal or high values for the various coagulation factors, low spontaneous fibrinolytic activity in the blood and a high content of FDP in serum and urine, thus changes not characteristic of consumption coagulopathy (Larsson et al.1971, Kleinknecht et al.1973)

In glomerulonephritis the basement membrane is known to be damaged after the antigen-antibody complexes are fixed to it. These complexes fix complement, and leukocytes accumulate. Enzymes are released from the granulocytes and may then cause local cell injury (Majno et al.1969,Janoff 1972,Colman 1974). It has been stressed that the granulocyte proteinases are injurious agents to tissue structures through their elastolytic and collagenolytic activity (Janoff and Zeligs 1968, Heimburger 1974,Cutz et al.1974). It is therefore possible that these proteinases released from the accumulated granulocytes in the glomeruli can cause further endothelial damage in glomerulonephritis. The proteinases may further

intensify the accumulation of granulocytes in the inflammatory process through cleavage of the complement factors C3 and C5 (Ohlsson and Olsson 1975).

Primary hemostasis and the coagulation mechanism can be initiated in several ways by the endothelial damage in glomerulonephritis. Müller-Berghaus and Eckhardt (1975) proposed that the granulocytes are of importance in the activation of the coagulation system and the precipitation of soluble fibrin in glomeruli. Lavelle et al. (1975) described recently a platelet aggregating factor released by sensitised leukocytes, which may be responsible for the platelet aggregates seen in glomeruli in glomerulonephritis. The collagen exposed by the endothelial damage may also initiate the platelet aggregation and activate the coagulation system resulting in fibrin deposition (Jaffe and Deykin 1975). Zimmerman and Müller-Eberhard (1971) showed, however, that the coagulation mechanism probably also might be activated directly, by activation of the complement system.

Our findings in 116 patients with recently discovered glomerulonephritis (paper I) speak in favour of such a local process confined to the kidneys associated with fibrin deposits. We thus found normal number of platelets, normal platelet adhesiveness, normal or high AHF-activity and AHF-related antigen, normal content of P & P and factor V, high values for fibrinogen, inhibitors of plasminogen activation, α_2-macroglobulin and negative ethanol gelation test. The spontaneous fibrinolytic activity in the blood was low. FDP were demonstrated in only 1/4 of the cases in the serum, but in practically all in the urine. The result of these coagulation and fibrinolytic studies in an early phase of glomerulonephritis thus produced no evidence of consumption of coagulation factors. No signs of consumption was either found during follow-up studies (up to 4 years).

It has been shown that the factor VIII protein is synthetised in the vessel intima and this protein has also been demonstrated in normal glomeruli of the kidney (Bloom et al.1973,Hoyer et al. 1973,-74, Holmberg et al.1974,Nachman and Jaffe 1975). The increased factor VIII levels in plasma in patients with glomerulonephritis and with gloomy prognosis, most probably reflect the degree of the glomerular endothelial vessel damage. Prentice et al.

(1972) and Vermylen et al.(1973) have shown that human platelets are aggregated in vitro by purified bovine and porcine high-molecular-weight factor VIII. They proposed that sialyl transferase in the platelet membrane complexes with exposed galactose residues in the factor VIII protein, which contributes to the aggregation of platelets. Besides the adhesion of the platelets to the collagen in the basement membrane, the increased AHF protein in glomerulonephritis might thus be of importance to initiate primary hemostasis and consequently fibrin deposition (Nachman and Jaffe 1975).

The break-down of the fibrin/fibrinogen related material in glomeruli requires the presence of fibrinolytic activity in glomeruli, which does not occur in healthy kidneys (Pandolfi et al.1972). It has been shown that glomeruli become fibrinolytically active in inflammatory conditions and in rejection episodes (Pandolfi et al.1972). This was confirmed in paper III in the investigation of cortical renal tissue with the histochemical technique.

What is it that initiates the fibrinolytic activity in glomerulonephritis?

In animal experiments glomerular fibrinolytic activity has been induced by coagulation and precipitation of fibrin in the kidney (Humair et al.1969,Bernik 1973). A good correlation between the local fibrinolytic activity in the kidney and the occurrence of fibrin deposits was also found in paper III. In the same paper (III) a good correlation was obtained between the degree of fibrin deposits and the occurrence of urinary FDP. These findings strengthen the assumption that urinary FDP may be the result of local lysis of glomerular fibrin/fibrinogen mediated by the fibrinolytic activators in the kidneys. It was recently stressed that granulocyte proteases also possess fibrinolytic activity which also may be of importance in the degradation of the accumulated fibrin/fibrinogen in glomerulonephritis (Ohlsson and Olsson.1975).

Urinary FDP may, however, also appear in the urine by filtration from the blood, or further degradation in the lower urinary tract of fibrinogen filtered through damaged glomeruli (Naish et al.1970, Clarkson et al.1971,Hedner 1974). Several authors have found no correlation between the presence of concentration of FDP in serum

and in urine (Hedner and Nilsson 1971,Larsson et al.1971,Preston et al.1971,Ekert et al.1972,Donati et al.1974). Only 31 patients in I, one of the patients in III and none of the patients in IV had FDP in serum, although almost all of them had FDP in the urine. Furthermore,the concentration of the urinary FDP was independent of the severity of the proteinuria and its selectivity (I,II,IV), which has also been stressed by Clarkson et al.(1971) and Vermylen et al.(1972). This suggests that urinary FDP are not due solely to filtration of serum FDP in glomerulonephritis with intact renal function. But in the presence of severe injury of the basement membrane filtration of FDP from the blood cannot be excluded (Wardle and Kerr 1975).

Further degradation of fibrinogen through damaged glomeruli requires the presence of urokinase in the urine (Boomgaard et al.1969). In patients with healthy kidneys the concentration of urokinase in the urine is high, while a decrease in the urokinase activity in the urine occurs in renal insufficiency (Vreeken et al.1966), but appears to occur also early in glomerular diseases (IV). The patients with early glomerulonephritis in paper IV had a low urokinase activity in the urine and these patients had all FDP of high molecular weight type, while 1 of the patients had low molecular weight degradation products (D and E). This patient had a normal urokinase activity and further degradation of the HMWDP in the lower urinary tract may therefore have occurred. All of the patients with early detected glomerulonephritis in papers I and II had a low concentration of urokinase in the urine, which further suggests that degradation of filtered fibrinogen in the lower urinary tract is unlikely in these conditions. In the presence of a severe damage to the basement membrane leakage of fibrinogen from blood cannot, however, be excluded, which has been demonstrated by Donati et al.(1974) by agarose gel chromatography.

It thus seems as if local glomerular lysis of fibrin is the most important mechanism for the appearance of urinary FDP, even if filtration of partially degraded fibrin/fibrinogen cannot be excluded in patients with severe damage to the basement membrane. A high fibrinolytic glomerular capacity can favour dissolution of these fibrin deposits and thereby improve the course of the disease.

The amount of FDP in the urine has been said to reflect the activity of the disease in glomerulonephritis (Stiehm and Trygstad 1969,Clarkson et al.1971,Dotremont et al.1973). To evaluate its significance, however, one must take into account the amount of fibrin deposited in the glomeruli and the glomerular fibrinolytic activity, which must also be of value for judging the prognosis as well as for deciding upon active treatment in a given case. It is obvious that definite conclusions from occasional determinations of FDP cannot be drawn but one can follow the course of the disease by serial determinations of FDP in the urine (VI,VII).

It was demonstrated in paper I that determining urinary FDP in glomerulonephritis was of no prognostic value. It appears that in early stages of glomerulonephritis factor VIII protein is of significance for judging the prognosis of the disease (papers I,II). The patients with newly discovered glomerulonephritis in I and II, who made a complete recovery during within 1/2 to 4 years, had, on admission, a normal factor VIII activity and factor VIII-related antigen. High factor VIII levels were on the contrary found initially in those patients who later developed a definite renal impairment. Several patients who developed persisting albuminuria and/or hematuria but no impairment of renal function, also often had normal values for factor VIII. In those patients the follow-up may have been too short.

Which parameters should then decide whether active treatment is indicated in glomerulonephritis?

The answer is difficult partly owing to the tendency to spontaneous healing and partly to our lack of knowledge of the pathogenesis of the disease. Obviously one can often not judge the prognosis of the disease from the histopathologic picture, from the excretion of albumin in the urine, or from renal function at onset (II). When in the course of the disease cytostatic treatment should be started is also uncertain (Merrill 1974). In addition, long term follow-up of the effect of immunosuppressive treatment in large materials is doubtful. These series have been summarised by Dixon (1971) and Merrill (1974).
It must therefore be of value for us in these early stages of glomerulonephritis to be able to judge the prognosis of the disease

so that those cases which can be expected to heal spontaneously are not exposed to the risks of immunosuppressive treatment.

By determining factor VIII related antigen in an early stage of glomerulonephritis it appears as if one can judge the degree of vessel injury and the probability of the disease healing or progressing. Repeated determinations of urinary FDP seems not to be of any prognostic value in early glomerulonephritis but is of great value to follow the course of the disease and the effect of treatment (I,VI,VII).

There is no agreement on the role played by disseminated intravascular coagulation in the pathogenesis of HUS and TTP. Most authors claim that the low platelet counts are due to an increased consumption and deposition of platelets in the kidneys (Brain et al.1967,Katz et al.1971,George et al.1974) or in the spleen (Katz et al.1973). The decreased half-time of the platelets in 2 patients in paper VI sustain an increased consumption of platelets in HUS and TTP. Brain et al.(1967) and George et al.(1974) reported an accelerated ^{131}I-fibrinogen turnover in HUS. In our material normal values of fibrinogen were measured in almost all patients in paper VI, which speaks in favour of a consumption of this protein in this highly active disease.

Other coagulation factors are said to be high or normal in most reports in these conditions (Lieberman 1972). No detailed daily studies of the coagulation and fibrinolytic factors have earlier been performed throughout the disease. Such studies must be decisive for evaluating the role played by continuous consumption. Daily determinations of the various coagulation and fibrinolytic factors was performed in 5 patients who filled the criteria for HUS and in 2 for TTP (paper VI). Examinations of these patients on admission revealed normal or raised levels of factors V and VIII, P & P and fibrinogen. The ethanol gelation test was negative, but FDP in the serum and in the urine were demonstrated in high concentrations. These results are in agreement with local coagulation with deposition of fibrin in the kidneys, rather than disseminated intravascular coagulation as has been shown by Katz et al.(1971) and Lieberman (1972). That consumption of the coagulation factors might take place during the course of the disease, however, was

shown in case 3. In this patient the platelet count, the levels of fibrinogen, factor V, factor VIII and P & P decreased significantly but fibrinolysis was not increased. In association with this the patient became worse with supervention of fluctuating neurologic symptoms and cramps. It therefore appears as if dissemination of the intravascular coagulation might occur in certain conditions.

In HUS there are usually symptoms of only renal origin, but the syndrome can change to a disseminated picture with involvement of multiple organs, which suggests that HUS and TTP have the same pathogenesis but with different manifestations, which is illustrated by case 5 in paper VI.

The clinical condition in HUS is often fluctuating. In association with an impairment of the patients clinical condition and renal function, a rapid decrease was seen in the platelet count and increased urinary FDP. An increase in the platelet count and decrease of FDP in the urine were the first signs of improvement.

In order to prevent further deposition of fibrin in the kidneys several authors have used treatment with heparin in these conditions. But no increased survival of those treated has been demonstrated, as summarised by Lieberman (1972). In our material heparin was given in a low dose, mainly to prevent clotting in the Scribner shunts. It had no prolonging effect on the coagulation times, but heparin may prevent a continued activation of factor X. Patients with neurologic symptoms and signs of dissemination of the disease, however, were treated with heparin in higher dose in order to prolong the APTT to twice its initial value.

Three of the patients in group I and 2 in group II (paper VI) were treated with hemodialysis, which was started as soon as the diagnosis had been made. Nutrition could be controlled in this way and the general condition of the patients could be followed satisfactorily during the acute course. The possibility of hemodialysis also having some direct effect on any toxic factor and thereby a favourable effect on the course of the disease cannot be excluded. A further 5 patients were treated in the same way during a later period with complete restitution of renal function. In one case, where hemodialysis was not considered indicated immediately, the patient died from irreversible hypertension. She had widespread cortical necrosis of her kidneys (personal observation).

The dialysis were given almost daily and for 3-4 hours until the

diuresis had been controlled and the serum creatinine had fallen spontaneously. The heparin treatment was continued until the platelet count had become normal, FDP in the urine had disappeared, and renal function had become normal.

In renal transplantation platelets and fibrin occur in the graft already in an early phase postoperatively, which is probably due to the ischaemic injury (Antoine et al.1969,Carlsson et al.1970). Parallel to this urinary FDP can be demonstrated up to 12 weeks postoperatively in concentrated urine in patients with an uneventful course. Demonstrable urinary FDP after this period always indicated some sort of complication such as rejection or glomerulonephritis in the graft (paper V). Reappearance or increase in urinary FDP is an early sign of acute rejection (paper V),which has earlier been found by Antoine et al.(1969), Clarkson et al.(1970) and Naish et al.(1973). This is of special importance for diagnosing an acute rejection episode during the first postoperative days, when other rejection parameters, such as sodium excretion, diuresis, albuminuria, tenderness to palpation over the graft, can be difficult to judge (paper V). Persistence of urinary FDP after renal transplantation thus seems to indicate a bad or at least a less successful prognosis for the graft. This is heavily sustained by several of the transplanted patients now followed up to 6 years without any signs of complication.

The occurrence of FDP in the urine in an early stage of glomerulonephritis and after renal transplantation has been demonstrated with the aid of concentration of the daily output of urine, which method, however, is time-consuming and difficult to perform every day (Clarkson et al.1971,Ekert et al.1972,Hedner 1974). To avoid this procedure a radioimmunoassay has been devised (VII). FDP in the urine are determined as D products obtained after complete breakdown of the HMWDP with streptokinase in the presence of plasminogen. A specific antiserum against purified D products is used. This makes the method exclusively specific, and the method can demonstrate FDP down to a concentration of 5 ng/ml, which makes it extremely suitable for diagnostic purpose and for following patients with glomerulonephritis or after renal transplantation.

CONCLUSIONS

1. In patients with early glomerulonephritis normal or high levels of the coagulation and fibrinolytic factors were found initially and during follow-up. In addition, low fibrinolytic activity in the circulation, negative ethanol gelation test and above all urinary FDP were demonstrated. This speaks in favour of a local process in the kidneys with fibrin depositions and against a consumption coagulopathy. High levels of factor VIII, especially factor VIII related antigen initially were found to be correlated with a bad prognosis of the disease with development of renal impairment (Papers I and II).

2. Local fibrinolytic activity in renal cortex was found with a histochemical method in early glomerulonephritis (Paper III). The presence of fibrin in the kidneys was always associated with local fibrinolytic activity in the glomeruli and the occurrence of urinary FDP. It was also shown that patients with proliferative glomerulonephritis and selective glomerular proteinuria had high levels of urinary FDP (Paper IV). These products were found to be of high molecular weight provided that the urokinase activity in the urine was low. It thus seems to exist prerequisits for fibrin deposited in the kidneys to dissolve by local fibrinolytic activity resulting in the appearance of urinary FDP.

3. Urinary FDP were always demonstrated in concentrated 24 hr urine in renal transplanted patients with an uneventful course postoperatively for up to 12 weeks. Demonstrable urinary FDP after this period always indicated some sort of complication such as rejection or glomerulonephritis of the graft (Paper V). Repeated determinations of urinary FDP therefore is of importance in the follow-up of patients after renal transplantation. Abscence of urinary FDP in concentrated 24 hr urine in repeated examinations seems to indicate a good prognosis for the graft.

4. Daily determinations of the different coagulation and fibrinolytic factors in HUS have shown that disseminated intravascular coagulation, as a rule does not occur, but that it can develop in certain conditions during the course of the disease (Paper VI). Daily determinations of urinary FDP and platelets were the best way of following the course of the disease in HUS.

Treatment with hemodialysis, as fast as the diagnosis was made, was shown to be of importance. Delay in this treatment seems to set the patients in a worse position with greater risk for developing hypertension and renal cortical necrosis. Low dose heparin given locally prevents clotting of the Scribner shunts, but also inactivates factor X_a, which may be of benefit for the course of the disease. If clinical signs of dissemination of the disease appeared, higher doses of heparin were given resulting in a prolonged APTT, preventing effectively further fibrin deposition.

5. The described radioimmunoassay is a sensitive and specific method for determining urinary FDP in patients with early glomerulonephritis and normal renal function without macroscopic hematuria. FDP in the urine are determined as D products obtained after complete breakdown of the HMWDP. This makes the method specific and has not been used in similar methods earlier described.

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